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# Dentritic, Spinal and Mitochondrial Alternations in Alzheimer's Desease. A Preliminary Descriptive Study

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**Abstract**

Alzheimer's disease (AD) is a devastating neurodegenerative disorder characterized by cognitive impairment, affecting memory and associated with behavioral and mood changes. The pathophysiology of Alzheimer's disease involves a number of cellular and biochemical mechanisms.
In the present study we aimed to describe the morphological alterations of dendrites, dendritic spines, synapses and mitochondria in Alzheimer's disease. For the purpose of the study we examined different brain areas in twenty brains from Alzheimer's disease patients, and twenty age-matched individuals who died accidentally.
We found significant loss of dendritic branches and decrease of spinal density, changes of the synapses and severe alterations of the mitochondria.
The findings of the present study constitute the pathological background for the cognitive decline seen in Alzheimer's disease, and are in favour of a significant role of mitochondria in early stages of the pathogenesis of the disease.

**Keywords***:* Alzheimer's disease, dendritic pathology, morphological alterations, mitochondria, oxidative stress

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## Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disorder characterized by cognitive impairment, affecting memory and associated with behavioral and mood changes. The pathophysiology of Alzheimer's disease involves a number of cellular and biochemical mechanisms [1].

The neuropathological hallmarks of the disease are the neurofibrillary tangles and senile plaques [2, 3], however a severe neuronal loss [4,5], marked synaptic and

dendritic alterations [6-8], and changes at the ultrastructural level have been described.

The etiology of the disease is not well understood, however, it is hypothesized that AD is the phenomenological expression of various metabolic, neurochemical, and morphological alterations on the basis of a broad genetic background [9], that are causing synaptic damage and therefore cognitive decline [10].

A positive maternal family history of AD increases the risk of Alzheimer's disease, a fact that could suggest the involvement of the mitochondrial DNA. Mitochondrial dysfunction is also linked to oxidative stress, which can also play an important role in early pathogenetic stages [11,12].

**Material and Methods**

We studied the hippocampi, the auditory cortex, the visual cortex, Broca's area, the Insular cortex, the medial and lateral geniculate bodies, the superior and inferior colliculi, and the cerebellar cortex of 20 patients who suffered from Alzheimer's disease, eight women and twelve men, aged between 62-89 years, who fulfilled the clinical, neuropsychological and laboratory diagnostic criteria of Alzheimer's disease. We also studied the same brain areas from 20 control age-matched individuals with no previous history of neurological disorder and who died accidentally.

The research was carried out with full respect to the appropriate legislation of the Greek Democracy (ν. 2472/1997, 2819/2000, 2915/2001, 3235/2004, 3471 /2006), as is Clearly stated by the Committee for Research Deontology Principles of the Aristotle University of Thessaloniki.

The average autolysis time for all subjects was 8±2.9 hours.

Gross examination of the brains was performed by a neuropathologist who was blinded to the medical history. The brains after the excision from the skull were immediately immersed in a formaldehyde 10% fixing solution where they remained for at least 25 days. Then we excised small parts from the aforementioned brain areas which have been used for the Golgi method and electron microscopy. The specimens were immersed in a dilution of potassium dichromate (7 g of potassium dichromate and 1ml of formaldehyde 37% in 300 mL of tap water) at a temperature of 18 o C. They remained in that solution for 1 week and then they were immersed in an aqueous solution of 1% silver nitrate where they remained for 1 more week at a temperature of 15 o C in a photoprotected environment. Afterward, the specimens were embedded in low-melting paraffin, cut with a slicing microtome at thick sections at the range of 120μm, covered with entellan, and studied with a Carl Zeiss Axiostar plus light microscope. Further adjacent specimens were used for Nissl methylene blue staining.

**Cell selection criteria**

For each one of the brains, fifteen neuronal cells were selected. Neurons examined for quantitative alterations met the criteria set forth by Jacobs et al. (1997) that

request uniform staining of neuronal processes, absence of precipitated debris, good contrast between cells and background, and relatively uniform tissue thickness [13].

For purposes of randomization, all the cells that met the selection criteria were randomly pooled and every third neuron in the series was chosen.

**Neuronal tracing and dendritic quantification**

For every one of the cells, we captured a 30-second video at a magnification of 400× while the microscope table was moving at the standard velocity of 20 μm/s. The microscope stage was moving using a motorized XYZ microscope stage system (MLS203/MZS500-E-ThorLabs), with the movement on the Z-axis being controlled by the MZS500-E– Z-Axis Piezo Stage and Controller Kit, with the aim of the APC software provided by Thorlabs with a JogStep of 1 μm a Travel Range of 250 μm. The videos were analyzed in digital image sequences of 200 serial pictures, which were ultimately imported in Neuromantic application to trace the cells, quantifying them along x-, y- and z coordinates [14]. Each one of the selected cells was traced using the Neuromantic application. Neuronal tracing was carried out in the semi-automatic form by two different investigators and the average of these measurements was used for statistical analysis. The neuronal tracing started with the cell soma and moved onto the basilar dendrites and the apical shaft. Dendritic trees were quantitatively evaluated in a centrifugal manner for apical dendrites and basal dendrites according to Uylings et al. (1990) [15]. Dendrites arising from the cell soma are considered first-order segments, up to their first symmetrical bifurcation. Dendritic branches arising from first-order segments are considered second-order segments, in turn, up to their symmetrical bifurcation into third-order segments, and so on. When asymmetric branching is met during the neuronal tracing, the offspring dendritic branch, recognized by a qualitatively thinner diameter, is classified as a next order branch, whereas the parent dendrite would retain its order-level past the branching point.

**Dendritic measures**

The parameters measured were: total dendritic length (TDL), the total number of dendritic segments and terminal branches, total dendritic area (TDA), and total dendritic volume (TDV).

**Spine counts**

Spine counts were carried out at 500 pictures, which were taken with an AxioCam HR, at the standard magnification of 1000×, on an Axiostar Plus photomicroscope. Visible spines were counted on three segments of the dendritic field. The first segment, 20–30 μm in length, was located in a distance of 50 μm of cells soma, the second segment, 20–30 μm in length in 150 μm, and the third one, 20–30 μm in 250 μm from cells soma.

**Neuronal density**

We also measured the neuronal density in Nissl stained specimens, on 20 images randomly selected for each cerebellar area for each brain at a standard magnification of 20X, with the help of the cell counter plugin in Image J software.

**Electron Microscopy**

Small samples from the same brain areas (2 × 2 × 2 mm), were excised and immersed in Sotelo's fixing solution, composed of 1% paraformaldehyde, 2.5% glutaraldehyde in cacodylate buffer 0.1 M, adjusted at pH 7.35. Then they were postfixed by immersion in 1% osmium tetroxide for 30 min at room temperature and dehydrated in graded alcohol solutions and propylene oxide.

Thin sections were cut in a Reichert ultratome, contrasted with uranyl acetate and lead citrate, and studied in a Zeiss 9aS electron microscope.

We studied the morphology of the mitochondria, and the synapses and proceeded to morphometric estimations at electron microscope on micrographs of a standard magnification of 56.000x.

**Statistical Analysis**

For the statistical analysis of the findings and the plotting graphs, we used R Studio. All the data were stored in CSV files and presented for the statistical analysis as data frames. A Student's t-test was used to determine whether significant differences existed across the independent parameters from neurons between the groups of this study (significance was taken as $P<0.05$). A Pearson correlation test was carried out in order to figure out if there is any correlation between the autolysis time and Purkinje cell dendritic complexity variables.

## Results

**Total Dendritic Length**

The total dendritic length was significantly decreased in all brain areas in AD patients, compared to controls.

**Terminal branches**

Neurons from AD brain showed a significantly lower number of terminals branches compared to controls.

**Branch length and Branch Order**

The mean branch length and the maximum branch order were both significantly decreased in AD brains, which also showed a significantly lesser branch length, and maximum branch order in comparison to controls.

**Dendritic spines**

The density of the dendritic spines was reduced in AD brains in all brain areas, with the distal segments of the dendritic trees being predominantly affected.

**Neuronal density**

Nissl staining showed a marked decrease in neuronal density in all brain areas in Alzheimer's disease brains, in comparison to control ones.

**Electron Microscopy**

Electron microscopy revealed significant pathological alterations of the dendritic spines, the synapses, and the mitochondria. The density of dendritic spines was significantly lower in AD brains, and the remaining spines were decreased in size, containing large multivesicular bodies, dysmorphic spine apparatus, and mitochondria with marked morphological changes. Mitochondria were characterized by significant pleomorphism and variation in size and shape, with fragmentation of cristae, and accumulation of osmiophilic material.

## Discussion

Dendritic pathology and decrease of dendritic spine density are prominent phenomena in early cases of Alzheimer's disease, which correlate significantly with the progressive decline of the mental faculties. In previous studies we have described the pathological alterations of the dendrites and the dendritic spines in the prefrontal area of the cortex and the cerebellum [6-8]. In this study we attempted to describe the morphological alterations of the dendrites and the dendritic spines, quantifying them in different brain areas of Alzheimer's disease, applying Golgi staining and electron microscopy. The present study showed extensive dendritic, spinal, and mitochondrial changes in different brain areas in Alzheimer's disease, in comparison to age-matched control brains. Golgi method revealed a significant loss of dendritic branches, resulting in a decrease in the total dendritic length, and a substantial decrease in the density of dendritic spines. The distal dendritic branches were mainly affected.

Electron microscopy showed that the remaining spines were smaller in size, and containing large multivesicular bodies, dysmorphic spine apparatus, and mitochondria with marked morphological changes. Mitochondria also showed marked pleomorphism, fragmentation of cristae, and accumulation of osmiophilic material. Mitochondrial pathology was more prominent in dystrophic dendrites, and is related to the loss of dendritic branches, and pathological alterations of the dendritic spines. We could hypothesize that mitochondrial alterations may play a very important role in dendritic degeneration and the loss of dendritic spines and we should have thought that therapeutic strategies protecting the mitochondria may be beneficial in Alzheimer's disease.

The loss of dendritic branches and dendritic spines, in combination with the loss of synapses and the presence of dystrophic synapses, described in the present study constitute the morphological background of the cognitive decline in Alzheimer's disease patients.

Mitochondrial alterations have been reported in several neurodegenerative diseases [16-18] associated mostly with oxidative damage [19]. Oxidative stress is mostly associated with amyloid $\beta$ (A$\beta$) accumulation in the neocortex [20,21], playing, therefore, an important role in the pathogenetic mechanisms of Alzheimer's disease [22], since it is not only involved in damage to the proteins of NFT and the formation of senile plaques but also involves extensive damage to the cytoplasm of neuronal populations vulnerable to death during AD [23].

Mitochondrial DNA is characterized by lack of histones, and this renders them a vulnerable target to oxidative stress. A$\beta$ peptide may also increase the production of mitochondrial reactive oxygen species (ROS) [24], causing further impairment of mitochondrial function [25].

Mitochondrial dysfunction has been associated with energy crisis of the cell and excitotoxic cell death and is considered to be of substantial importance in the cascade of phenomena, which eventually lead to apoptosis. It is possible that mitochondrial dysfunction occurs early and acts causally in disease pathogenesis, as some observations in early cases of Alzheimer's disease [26] indicate that morphological alterations of the mitochondria and oxidative damage may be one of the earliest events in Alzheimer's disease. The morphological alteration of the mitochondria seen in AD brains, pleads in favor of a generalized mitochondrial dysfunction in Alzheimer's disease, which may be associated with wide neuronal loss and synaptic alterations, seriously affecting consequently, the mental faculties, which are basically related to extensive neural networks [27].

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